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Larval and Early Juvenile Development in *Tegula funebralis* (Adams, 1855) (Gastropoda: Trochidae) in Baja California Sur, México

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Abstract. Larval and early juvenile development in *Tegula funebralis* was observed for 63 days under static culture conditions at temperatures ranging from 19°C–20°C. The post-larvae were fed *Phaeodactylum trichornotum* microalgae. Embryonic development from fertilized egg *in vitro* to competent larval stage lasted 8 days. Teleoconch secretion occurred on day 11. Very fine longitudinal striations, appearing on the anterior edge of the teleoconch on day 14, became parallel ribs extending from the teleoconch to the protoconch in juvenile stages. The shell aperture acquired a fanlike shape and the dextrally coiled spire rose, becoming conical on day 48.

INTRODUCTION

Along the central Pacific coast Baja California, the community associated with abalone (*Haliotis* spp.) banks includes a variety of gastropod species. Most common, in terms of frequency and abundance, are *Megastraea* (= *Astraea*) *undosa* (Wood, 1828), *Megathura crenulata* (Sowerby, 1825), and species of the genus *Tegula* (Guzmán del Próo et al., 1991). *Tegula funebralis* is widely distributed in the intertidal zone of the Pacific coast of the Baja California Peninsula (Guzmán del Próo et al., 1991). Other species of this genus, *T. aureotincta* (Forbes, 1850), *T. eiseni* (Jordan, 1936), and *T. regina* (Stearns, 1892), share the habitat of *H. fulgens* in the subtidal zone of Bahía Tortugas (Carreón-Palau, 2000).

From recent studies on microhabitat and recruitment in juvenile *Haliotis* spp. (Carreón-Palau, 2000; Carreón-Palau et al., 2003) and some preliminary experiments on settlement of post-larval abalone in artificial collectors (Ponce-Díaz, 2004) in this area, the need to identify post-larval and early juvenile stages of the gastropods associated with the abalone rocky reefs has emerged. These species share a habitat and overlap in their spawning season, September to November (Belmar et al., 1991; Belmar & Guzmán del Próo, 1992; Guzmán del Próo, unpublished data).

Identification of these early life history stages has proved difficult because there is a dearth of literature on reproduction and development of members of the Trochidae (C. S. Hickman, quoted in Kulikova & Omel'yanenko, 2000). Consequently, in the laboratory, the authors have been culturing the major gastropods sharing the habitat of *Haliotis* spp. to obtain reference collections that will assist in identifying larval and early juvenile stages of these gastropods.

Larval development in *T. funebralis* (Adams, 1855) was described by Hewatt (1934) and later by Moran (1997) for a population in Oregon. This study presents results from a population in Baja California Sur, about 2500 km to the south. Here, females were induced to spawn. Detailed drawings of the development, which complements available information, will facilitate future identification of the early stages of this species in all of its range.

MATERIALS AND METHODS

Collection and Maintenance of Specimens

We collected 60 adult specimens of *T. funebralis* (basal diameters 22–26 mm, shell height 15–20 mm, and total weight 5.17–8.04 g). The specimens were collected in the rocky intertidal zone of Bahía Tortugas, B.C.S. (27.7°N, 114.9°W) in January 2002. The specimens were transported in a cooler, maintaining humid conditions by layering the specimens between folds of giant kelp (*Macrocystis pyrifera*) leaves with an interior temperature of 10°C.

In the CIBNOR laboratory, the specimens were placed in 40-L plastic aquariums. Seawater in the aquariums was kept between 18°C–20°C with constant aeration. The specimens were fed rehydrated giant kelp (*M. pyrifera*) leaves. Water and food were replaced every other day.

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Gonad Conditioning

To attain maximum gonad development, specimens were fed *ad libitum* and the water kept between 18°C–20°C. Five snails were sacrificed weekly to monitor the stage of gonadal maturation by microscopic observations of gametes. Color of gonads (cream in males and moss green in females) provided determination of gender.

Induction of Spawning

After 60 days of gonadal conditioning, spawning was induced. Fifteen snails were removed from the aquarium (about 20°C) and were exposed to air for 30 min at 19°C in the shade and for 15 min at 29°C in the sunlight. They were then subjected to abrupt temperature shifts in seawater: the snails were returned to an aquarium at 16°C, then to an aquarium at 25°C. The cold and warm treatment was done twice, holding the specimens in each aquarium for 30 min. The snails were then returned to the 40-L aquarium. About 24 hr after thermal stimulation, only unfertilized eggs were found on the bottom of the aquarium. These eggs were fertilized with sperm extracted form dissected males (1 oocyte/15 sperm).

Sieving of Embryos and Larvae

Embryos were cultured in 16-L tanks and benthic phase specimens in 20 × 30 × 5 cm trays. Seawater was passed through a filter (1 μm pore size). Larvae were sieved through Nytex mesh (236, 160, 140, and 100 μm pore size) to remove organic waste. Sieving was done every 48 hr throughout the experiment. Benthic post-larvae were fed *Phaeodactylum tricornotum* (Bohlin, 1974) microalga. A video monitor attached to a microscope was used to observe and record morphological changed associated with larval and early juvenile development through day 63. Drawings were made from video images by tracing the outline and the main features of each stage directly from the TV screen. Mean (±SD) length of all stages was based on a sample of five individuals at each stage. From the veliger stage onward, size refers to the long axis of the shell. Seawater in the aquariums was kept at 19°C–20°C during growth phases through day 63.

RESULTS

Removal and maintenance of adult *T. funebralis* in coolers with a humid environment proved effective. No deaths occurred following 14 hr of transport. In the laboratory, the specimens ate the food supplied (rehydrated *M pyrifera*) as their regular diet throughout the experiment.

Induced spawning had positive results in females, while males failed to spawn. *In vitro* fertilization was successful, allowing subsequent observation of larval development. Observations were made from the time of fertilization through the early juvenile phase. The following descriptions identify the stages and more prominent features during early development of the species (Figure 1).

Embryonic Development

Day 1. Fertilized eggs range in diameter, 145 ± 5 μm. Eggs are enclosed in a membrane with outside diameter 175 ± 5 μm, with a thick, additional gelatinous cover 140–320 μm thick (1). Eggs are bright green, remaining so throughout larval development. First cleavage occurs at 35–45 min, resulting in two same-sized cells (2). Second cleavage occurs at 55–65 min, forming four cells (3). Third cleavage occurs at 1–2 hr, forming eight cells. Subsequent cleavages occur after 2 hr (4). The ciliated gastrula (5–6 hr) remain enclosed in the egg membrane, attaining a diameter of 155 ± 15 μm diameter (5). Invagination of the posterior end occurs (6), which corresponds to the shell gland. Trochophore forms at 9–10 hr, reaching a length of 160 ± 10 μm (7). After elongation, prototrochal girdle begins to develop at one end (8) and two lateral tufts of cilia appear on its base (9). Early veligers form at 21–22 hr and are 200 ± 10 μm long (10). Velum (11) and primordium of the foot (12) are present. Shell covers the entire body (13) except the velum.

Day 2. Veliger larvae after torsion, 223 ± 16 μm long (14). Cephalo-pedal mass (15) with operculum (16). The velum branches into two sections (17) and lengthens posteriorly to form cephalic tentacles. Foot displays retractile movements and larvae swim with irregular motions.

Days 4–7. Late veligers are 226 ± 10 μm long (18). Larvae withdraw into shell. Formation of the first whorl occurs. Eye spots are apparent (19). Shedding of cilia begins (20). Larvae exhibit exploratory movements in search of attachment substrates.

Benthic Phase

Day 8. Post-larvae are 240 ± 12 μm (21a). Cephalic tentacles (22) and mouth (23) appear, and operculum becomes prominent (24). A few remnants of cilia tufts remain but with little motility (25). All larvae have settled.

Day 11. Post-larvae are 255 ± 10 μm long (21b). When the first suture forms, it separates the protoconch from the teleoconch, which now begins to develop (26).

Juveniles

Day 14. Juveniles are 271 ± 10 μm long (27a). Very fine longitudinal striations start to form on the anterior edge of the teleoconch (28).

Day 22. Juveniles are 290 ± 20 μm long (27b). Longitudinal striations become more conspicuous and take the shape of ribs running the length of the shell (29). Transverse sutures in the teleoconch increase (30). Shell spiral

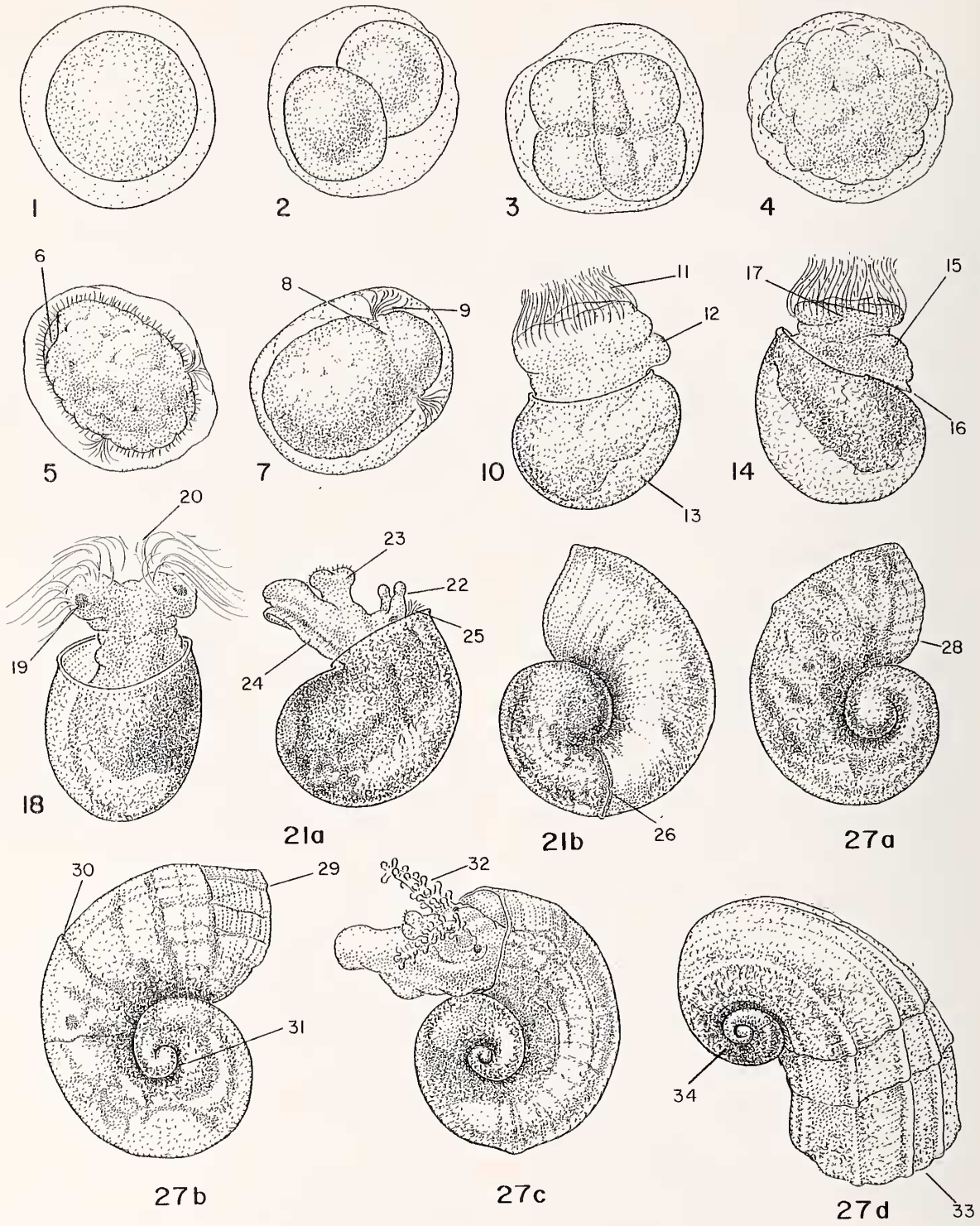

Figure 1. Developmental stages of *Tegula funebralis* from egg to 48 days. (**1**) Fertilized egg. (**2**) First cleavage. (**3**) Second cleavage. (**4**) Morula. (**5**) Gastrula. (6) Shell gland. (**7**) Embryonic trochophore. (8) Proto-trochal girdle. (9) Lateral cilia. (**10**) Early veliger. (11) Velum. (12) Foot primordium. (13) Shell. (**14**) Veliger after torsion. (15) Cephalo-pedal mass. (16) Operculum. (17) Branching of velum. (**18**) Late veliger. (19) Eye spots. (20) Shedding of velum. (**21a,b**) Post-larvae. (22) Cephalic tentacles. (23) Mouth. (24) Operculum. (25) Remnant cilia. (26) First suture. (**27a,b,c,d**) Juvenile. (28) Longitudinal striations. (29) Ribs. (30) Transverse sutures. (31) Spire. (32) Cephalic tentacles. (33) Shell aperture. (34) Dextrally coiled shell. Bold numbers = different stages, normal numbers = morphological characters.

Table 1.

Development time in *Tegula funebralis* from embryo to juvenile under laboratory conditions with temperature range of 19°C–20°C.

Stage	Time
First cleavage (two cells)	35–45 min
Second cleavage (four cells)	55–65 min
Third cleavage (eight cells)	1–2 h
Ciliated gastrula	5–6 h
Trochophore	9–10 h
Early veliger	21–22 h
Veliger	2 days
Late veliger	4–7 days
Settlement (benthic phase), two cephalic tentacles	8 days
Postlarva: suture separates proto-conch from teleoconch	11 days
Juvenile: fine longitudinal striations	14 days
Juvenile: ribs running the length of the shell, cephalic tentacles with 12 papillae	22 days
Juvenile: Cephalic tentacles with 18 papillae	34 days
Juvenile: Cephalic tentacles with 21 papillae	43 days
Conical shell	48 days

is more evident (31). Cephalic tentacles have 12 papillae each.

Day 34. Juveniles are 380 ± 60 μm long (27c). Longitudinal ribs and transverse sutures become very evident. Cephalic tentacles have 18 papillae (32).

Day 43. Juveniles attain length of 455 ± 30 μm; cephalic tentacles have 21 papillae each.

Day 48. Juveniles are 463 ± 9 μ long (27d). Shell aperture is now fan-shaped (33); dextrally coiled spire rises, becoming conical (34). Juvenile appearance remains unchanged from this day through day 63, when the experiment ended. Table 1 and Figure 1 summarize the stages of development. Figure 2 summarizes growth during the experiment. The rate of growth was about 5.6 μm day^{-1}.

DISCUSSION

Maintenance of *T. funebralis* under laboratory conditions posed no problems. Specimens adapted easily to feeding on rehydrated *M. pyrifera* foliage. The induction to spawn through different methods (thermal shock, desiccation, hydrogen peroxide, UV-radiation of seawater, etc.) has been used in gastropods (Kikuchi & Uki, 1974; Holyoak, 1988; González et al., 1999; Leighton, 2000). In this study, although different temperatures and times to spawning induction were assayed, it was possible to obtain the spawning of females only. This species is naturally adapted to drastic thermal changes in their environment, the intertidal zone, which may be related to its limited response to changes in temperature.

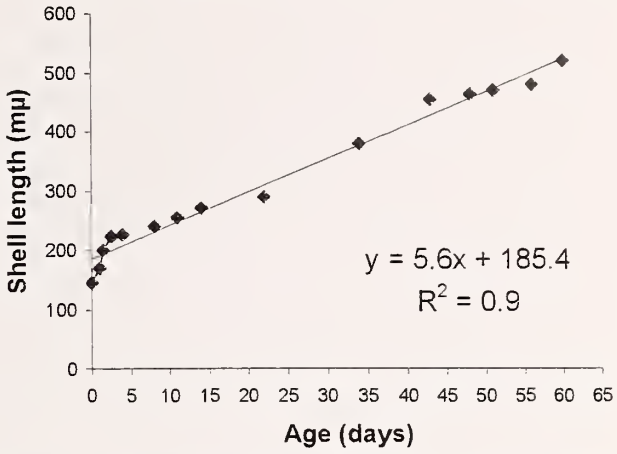

Figure 2. Post-larval and juvenile growth of *Tegula funebralis*. Day 0 corresponding to veliger stage. ♦ = average of 5 measurements.

Release of oocytes was an important factor for the success of the experiment because of attempts to obtain oocytes through gonadal dissections failed. Female gamets have very fragile membranes that break when manipulated for artificial fertilization. Oocytes released into the water suggest that fertilization is external, agreeing with observations by Moran (1997), who rejected a process of internal fertilization reported years ago by Hewatt (1934).

Embryonic development up to the formation of the trochophore took 9–10 hr. Moran (1997) reported 25 hr for this species. Kulikova & Omel'yanenko (2000) reported 16–17 hr for *T. rustica*. From veliger larvae to the onset of the benthic phase, development time was similar to the report of Moran (1997), the larvae attaching between days 6–8. Moran noted longitudinal striations on the shell on day 21, while our observations placed this feature at day 14. Another important difference is the absence of pigmentation in the foot and head in our specimens, compared to Moran's finding of pigmentation in juveniles at 2 months' development.

Such variations in *T. funebralis* may be a consequence of temperature conditions during the experiment: 18–20°C in our study and 13–15°C in Moran's investigation. For *T. rustica*, differences could be related to species characteristics, since early development in this species took place at temperatures similar to those used in our study.

From day 14 onward, some events and features, such as transverse sutures in the teleoconch, number of papillae, shell-aperture shape, spire-raising, and time of adult characteristics, cannot be compared with Moran's study because they were not described in his work.

We found that larval development among the species that we studied is very similar until the veliger stage. To distinguish conspicuous differences, experiments need to be undertaken beyond that stage, preferably to the early

juvenile stages, where teleoconch development begins to show different morphological characteristics. For example, in early juvenile *T. funebralis*, very fine longitudinal striations are formed on the anterior edge of the teleoconch. These become longitudinal ribs in juveniles. In *Megastraea undosa*, early juveniles develop a crenulated ornamentation and brown spots at the edge of the teleoconch (Guzmán del Próo et al., 2003).

The recognition of these differences among early juvenile stages of gastropods living in rocky habitats, in association with abalone (*Haliotis* spp.), will provide important information about intensity of reproductive activity and settlement patterns shared by these species and the dynamics of interaction within the rocky communities of the central Pacific coast of Baja California.

Understanding reproductive interactions and strategies that these species have developed for resource allocation may help improve management of heavily exploited species, such as *Haliotis* spp. and *M. undosa*, as well as of other species associated with this benthic community that may come under future management by reason of their commercial value.

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